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<b>13. ABSTRACT (Maximum 200 Words)</b> A significant proportion of human breast cancers overexpress ErbB2, a member of the receptor tyrosine kinase gene family that also includes the epidermal growth factor receptor (EGFR). Overexpression of ErbB2 correlates with increased metastatic potential and poorer prognosis. Agents that antagonize the activity of ErbB family members have obvious clinical implications. We have previously discovered that decorin causes a functional inactivation of the oncogenic ErbB2 in mammary carcinoma cells overexpressing ErbB2. This leads to growth inhibition and cytodifferentiation of mammary tumor cells and a concurrent suppression of their tumorigenic potential in vivo. We have successfully demonstrated decorin's cytostatic effect both in vitro and in vivo with a metastatic breast cancer cell model. Thus, decorin gene therapy helps in retarding the growth of human tumors in immunocompromised animals and could represent a new independent or adjuvative therapeutic modality against cancer. We have additionally created a breast cancer cell line that contains the decorin transgene under the control of an inducible promoter. We plan on using this cell line to study decorin's temporal effects on both primary tumor development as well as on tumor spread and metastases. Our ultimate goal is to prove decorin's efficacy as a tumor suppressor and possible means of therapy for breast cancer.				
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### INTRODUCTION

A significant proportion of human breast cancers overexpress ErbB2, a member of the receptor tyrosine kinase gene family that also includes the epidermal growth factor receptor (EGFR). Overexpression of ErbB2 correlates with increased metastatic potential and poorer prognosis. Agents that antagonize the activity of ErbB family members have obvious clinical implications. We have previously demonstrated that decorin is a novel ligand for the EGFR. This interaction triggers a signaling cascade that leads to activation of MAP kinase and ultimately to growth suppression. In the preliminary data that support the basis of this proposal, we discovered that decorin causes a functional inactivation of the oncogenic ErbB2 protein in mammary carcinoma cells overexpressing ErbB2. This leads to growth inhibition and cytodifferentiation of mammary tumor cells and a concurrent suppression of their tumorigenic potential *in vivo*. We hypothesize that expression of decorin by breast carcinoma cells in an *in vivo* tumor model will result in inhibition of tumor growth. We further hypothesize that exogenous administration of the human decorin transgene via a viral or liposomal transfer vector will lead to growth slowdown and/or growth inhibition of already established xenograft tumors of human breast carcinomas.

### BODY

We have made significant progress in several areas, including finding a suitable breast cancer model, treatment of the model *in vitro* and *in vivo* with adenoviral decorin, and in the production of a cell line containing decorin under the control of an inducible promoter.

#### Breast cancer tumor model

We have tested several cell lines for tumorigenic potential in mice and have decided to use the MTLn3 cell line (a kind gift from J.E. Segall, Albert Einstein Medical Center). The MTLn3 cell line is a rat breast adenocarcinoma cell line that can spontaneously metastasize *in vivo* to the lung, much like human breast cancers, and expresses moderate amounts of EGFR and high amounts of ErbB2 (~500,000 receptors/cell).

#### Inducible decorin promoter

We have created MTLn3 cells expressing decorin under the control of a Tet responsive element with the RevTet On System (Clontech). MTLn3 cell clones express decorin only in the presence of doxycycline, a harmless antibiotic. We have confirmed high levels of inducible decorin expression in several clones via Western blotting (Figure 1). Growth inhibition tests *in vitro* are pending, as is Western blotting to confirm the amounts and phosphorylation states of EGFR and ErbB-2. *In vivo* tests will begin shortly if results are favorable.

#### Adenoviral decorin vector

The MTLn3 cell line has been successfully growth-inhibited *in vitro* using adenoviral decorin. Treatment of MTLn3 cells with adenovirus expressing decorin resulted in approximately 50% growth inhibition after 72 hours in culture with MOI's of as little as 0.1 (1 viral particle for every 10 cells). Decorin's secretion into the local environment is a major asset in these experiments, as all nearby

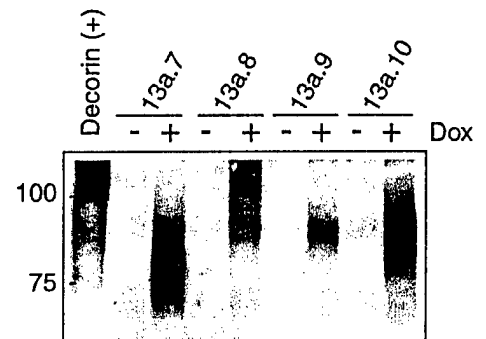


Figure 1. Induction of decorin in MTLn3 cells. Western blot of media harvested from cells after induction with doxycycline (5µg/ml).

cells can be affected from decorin, not only those cells successfully transduced. We have additionally treated MTLn3 tumors *in vivo* in nu/nu and SCID mice with adenoviral decorin. Tumor growth inhibition of 70% was observed after only two treatments of adenoviral decorin ( $5 \times 10^8$  pfu per treatment) following tumor induction directly at the breast with  $10^6$  MTLn3 cells (Figure 2). We have previously shown this adenoviral vector to be successful in treating other types of tumors *in vivo* in preliminary testing (Reed et al., 2002).

#### AAV decorin vector

Generation of an AAV vector containing decorin is still a priority. AAV (adeno-associated virus) is a powerful viral delivery method for gene therapy (During 1997, Samulski et al., 1999). We are looking into the AAV Helper Free System (Stratagene). The system employs the AAV2 serotype virus, which can use either of two receptors for viral entry into cells, making it more versatile than other serotypes. The system additionally features a  $\beta$ -globin intron to improve message lifetime in the cytoplasm. If this system looks promising, then we will clone decorin into the packaging plasmid and begin *in vitro* testing.

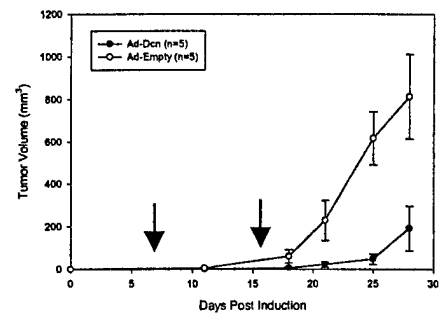


Figure 2. Ad-Dcn inhibits the growth of MTLn3-induced tumors *in vivo*. Graph showing growth inhibition of MTLn3 tumors in nu/nu mice. Ad-Dcn treatments indicated by arrows.

#### KEY RESEARCH ACCOMPLISHMENTS

- Use of MTLn3 breast carcinoma tumor model to generate tumors *in vivo* that spontaneously metastasize to the lungs.
- Successful treatment of MTLn3 tumors *in vivo* with adenoviral decorin.
- Creation of MTLn3 cell clones with decorin under the control of an inducible promoter (Tet On System).

#### REPORTABLE OUTCOMES

At this time there are no reportable outcomes.

#### CONCLUSIONS

The ultimate goal of this research is to prove that human decorin is a viable candidate or adjunctive candidate for treatment of certain breast cancers, and that it is possible to deliver the decorin transgene by one or more well-established transfer methods to achieve a positive clinical response. While we have experienced considerable success with adenoviral vectors containing decorin (Reed et al., 2002, and unpublished results), our ultimate goal is to produce a long-lasting expression vector that also is less immunogenic. AAV viral vectors meet both criteria, but our attempts with it unfortunately suffer from low levels of expression. Our current strategy, an improved AAV-2 vector containing a  $\beta$ -globin intron and having the ability to package larger amounts of DNA, should prove

advantageous in the near future. Meanwhile, we will continue our work with the MTLn3 tumor model and adenoviral decorin vector, looking further into metastatic spread and prevention or reduction thereof. Additionally, our inducible cell line is being prepared for *in vivo* testing shortly, allowing us to assess the temporal effect of decorin expression on breast cancer development and spread. We have already been able to show that our concept- that decorin has promise as a future candidate in the battery of treatments against breast cancer- and we hope to be able to go on to solve the problems of a superior delivery system. We also are planning on exploring the temporal effects of decorin expression on tumor growth and tumor metastasis, a serious problem in breast cancer, with our inducible promoter. Decorin has so far proven to have powerful antitumor properties while completely lacking in toxicity, an important point to keep in mind. The next several months have great promise.

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## APPENDIX

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